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RELATION OF FEEDING IN THE SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS* TO DIVISION IN SOME OF ITS ENDOCOMMENSAL CILIATES¹

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Seven species of ciliates, which are generally regarded as commensals, have been reported from the digestive tract of *Strongylocentrotus droebachiensis* in the Mt. Desert Island region (Powers, 1933a; Beers, 1948). Four of them (*Cyclidium stercoris*, *Plagiopyla minuta*, *Euplotes balteatus*, and *Trichodina* sp.) are of erratic occurrence and are excluded from the present study, which is therefore based on the remaining three, namely, the holotrichs *Entodiscus borealis* (Hentschel), *Madsenia indomita* (Madsen), and *Biggaria gracilis* (Powers). They are of almost invariable occurrence in any urchin whose test exceeds 10 mm. in diameter, and the number of individuals of any one of them in an urchin 25 mm. in diameter or larger may be enormous. They were present in all the urchins of the present study.

Although the food of the three has not been studied critically, the evidence indicates that it is largely bacterial. In some gastroles of *E. borealis*, Powers (1933b, p. 129) reported "rod-like bodies which resemble bacteria," although in others he noted objects which look like the nuclei of intestinal epithelium cells. In *B. gracilis* he reported "bacteria and bits of algae" (1933a, p. 112), but the nature of the food of *M. indomita* was unmentioned. I have observed rod-like structures and short filaments, which were undoubtedly bacteria, in the gastroles of all three ciliates, but some unidentified material was also present.

Various aspects of the autecology of the ciliates have been treated elsewhere (Beers, 1948, 1961). Findings pertinent to the present study and reported in 1948 may be summarized as follows. *Biggaria gracilis* is essentially an inhabitant of the rectum, which, except in drastically starved urchins, always contains food remnants. In the usual adequately fed urchin *E. borealis* occurs primarily in the stomach and *M. indomita* in the intestine. The regions of the gut being ill-defined, the distribution of the ciliates is not a rigid one. If an urchin is kept without food for a week or longer, the stomach and intestine gradually become empty and the distribution of *E. borealis* and *M. indomita* changes, in that they shift toward the rectum, where they mingle with *B. gracilis*. Dividing specimens of *B. gracilis* can be found in

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practically any urchin that is collected in nature, but it is a remarkable fact that dividing specimens of *E. borealis* and *M. indomita* are extremely difficult to find, even in ciliate populations of great density. Of 182 urchins that contained immense numbers of both ciliates, only six had dividing specimens of *E. borealis* and only three those of *M. indomita*. (Powers, 1933b, likewise had difficulty in finding dividing forms of *E. borealis* and indeed found only three in all his material, which was evidently plentiful.) It was concluded that division occurs cyclically in the two ciliates: that "short periods of intense divisional activity . . . alternate with long periods of non-divisional life" (p. 111). Since the urchins appeared to be adequately fed and to contain ample bacteria to support the ciliates, the conclusion implies that the cycles are inherent in the ciliates.

I was never entirely satisfied with the conclusion, for cycles of the kind postulated are generally absent in ciliates. When interruptions of division occur, they are usually associated with environmental inadequacies or with special physiological states, such as senescence, autogamy, conjugation, or the production of resting cysts. Since the feeding habits of the urchins were uncontrolled in my earlier study, I returned to the subject in the summer of 1962, in an effort to ascertain whether the division of the ciliates is in any way related to the food of the host. More precisely, the study concerns the following questions, the first two of which are somewhat preliminary, whereas the third is the principal one. (1) What is the condition of the ciliates with respect to division in urchins that have been deprived of food for a considerable period, meaning about two weeks? Earlier observations (unpublished) indicated that dividing forms of *E. borealis* and *M. indomita* are absent in such urchins, but the condition of *B. gracilis* was unrecorded in my notes. (2) Hence, are dividing forms of *B. gracilis* also absent? It is logical to expect a cessation of division in the absence of food, and this expectation was readily confirmed. (3) Then, if these starved urchins are again supplied with ample food, is division resumed in *B. gracilis* and is a cycle of division initiated in *E. borealis* and *M. indomita*?

MATERIALS AND METHODS

From time to time during the summer, specimens of *S. droebachiensis* were collected at low tide from the rocks and shallow water near the Mt. Desert Island Laboratory. In size they varied from 25–70 mm. in diameter (test, excluding spines); most of them measured 40–55 mm. across. Injured specimens (spines damaged or integument abraded) were rejected, for these may be attacked and eaten by healthy ones. The urchins were transferred in groups of 60–70 to aquaria through which sea water flowed constantly. In these, they were kept without food for two weeks or longer (usually 14–16 days). Voided fecal pellets, which may be re-ingested, were removed daily by siphoning.

Several representative urchins (usually 5 to 10) of each group were opened and examined as soon as collected, and the condition of their ciliates was recorded. In all the examinations of the entire study the relative abundance of each ciliate in the stomach, intestine, and rectum was estimated as formerly and the respective densities of population were recorded as "light," "moderate," or "heavy" (Beers, 1948). If dividing individuals of a particular species were present, the number of such individuals, as well as the total number, was counted in samples containing

50–150 ciliates of the species. From these totals the percentage of dividing individuals in the samples was calculated and this figure was assumed to represent conditions in the urchin. The presence of an occasional dividing specimen was ignored. But if one specimen or a larger number was dividing in every 100 individuals of the species, division was judged to be significant and the appropriate percentage was recorded.

After two weeks of starvation, several urchins of each group were again examined and the condition of their ciliates was recorded. Then, smaller numbers of starved urchins (usually 10 to 20) were removed to other aquaria and were supplied with pieces of healthy fronds of the kelp *Laminaria* sp., which is a preferred food of *S. droebachiensis* and is indeed adequate, as Swan (1961) demonstrated, to maintain it in a healthy, growing condition for at least a year. Usually the urchins were placed directly on, or in contact with, a piece of kelp; thus, they sensed its presence at once and began to feed without delay. Since an excess of kelp was maintained in the aquaria, ample food was always available to the urchins. These laminaria-fed urchins were then opened and examined at various hourly intervals following the addition of food. By varying the time of day at which laminaria was initially supplied, examinations could be conveniently planned for any sequence of hourly intervals within the period of experimental feeding, which was arbitrarily restricted to 5 days. By using sufficient numbers of urchins, nearly all the hourly intervals of the 5-day period were finally represented in the results. It is true that the number of urchins examined for any particular hour was relatively small (usually two to five), since the examination of an urchin requires 15–45 minutes, depending on the condition of its ciliates. But the total number of such urchins was considerable and amounted finally to 294.

RESULTS

1. Condition of the ciliates in urchins collected in nature

Thirty-two urchins taken from the various collections and examined immediately showed the usual ciliate distribution and population densities (some "light," but many "moderate" to "heavy"), in agreement with earlier observations (Beers, 1948). Whereas *B. gracilis* was dividing in all the urchins, only one contained dividing specimens of *E. borealis*, and another those of *M. indomita*, though division in each case was sparse. The digestive tracts of the urchins appeared to contain ample food, chiefly masses of filamentous green algae and fragments of laminaria. These observations indicated that the urchins which were subjected to starvation were healthy, adequately fed, and typical with respect to ciliates; that *B. gracilis* was dividing in all of them; but that only about 3% of them contained dividing specimens of *E. borealis*, and a like percentage those of *M. indomita*.

2. Condition of the ciliates in starved urchins

Thirty-five urchins from various experimental groups were examined after 14–21 days of starvation. In all of them the stomach and the greater part of the intestine were empty (lacked solid food); only the terminal quarter of the intestine and the rectum contained some undigested or indigestible material, but the

amount was small. *Entodiscus borealis* was present in the intestine and rectum, as well as the stomach, and *M. indomita* was present in the rectum, as well as the intestine. Evidently these ciliates, as digestion proceeded, had dispersed aborally from their preferred sites. In accordance with expectations, dividing specimens of the two were absent and population densities were reduced to "moderate" or "light." (All urchin ciliates escape regularly in limited numbers among the fecal pellets; in the absence of division, reduced numbers are the rule.) As usual, *B. gracilis* was restricted to the rectum, but dividing specimens were absent in all the urchins except one, in which only two such specimens could be found. Microscopic examination of representatives of the three species showed that the cytoplasm was very transparent and contained relatively few gastroles. Evidently a two-week period of starvation of the host was adequate to reduce the division rate of all the ciliates practically to zero, and it was concluded that the starved urchins contained to all practical purposes no dividing ciliates.

3. Feeding habits of starved urchins

The starved urchins fed readily on laminaria. The stomach was well filled after 6–8 hours of feeding; the stomach and intestine after 15–18 hours (counting from the beginning); and the stomach, intestine, and rectum after about 20 hours. Feeding continued (by night as well as by day) for 60–72 hours, when many of the urchins evidently became surfeited and moved away from the food. On the fourth and fifth days of the experiment, some urchins were always feeding, while others rested near-by on the sides of the aquarium. Thus, feeding occurred irregularly on these days. Some of the urchins were kept a total of 6 to 14 days with the laminaria—well beyond the end of the formal experimental period. These urchins also fed irregularly, probably as urchins feed under natural conditions. The intestine and rectum were always well filled, but the amount of food in the stomach was variable.

4. Condition of the ciliates in urchins fed laminaria after two weeks without food

The results of the feeding experiments are summarized in Table I. It is understood that this table is a composite of all experiments, rather than a continuous record of a single vast experiment. The table concerns 294 urchins (total of Column 2), and it is obviously impossible for one investigator to start with this number of starved urchins and to continue the examinations uninterruptedly for 120 hours. In the table the successive hours are grouped by 8-hour periods, and for reference purposes the periods are numbered (Column 1). It is important to note that all the urchins of any period are represented in each of the three columns under "Number (and percentage) of urchins. . . ." A consideration of some of the periods will clarify the method of presentation. When two paragraphs appear under a period in the following account, the first deals with results presented in the table, and the second contains explanatory or supplementary comments.

Period 1. There was examined a total of 20 urchins (Column 2) that had fed from 1 hour to 8 hours on laminaria (Column 3). Since no dividing ciliates could be found in any of these urchins, it follows that the remaining columns of the period read zero.

Period 2. In this period 30 urchins that had fed from 9 to 16 hours were examined. In 18 of the 30, or 60% of them, *E. borealis* was dividing (Column 4), but dividing specimens were absent in the remaining ciliates.

The number of dividing specimens of *E. borealis* in the 18 urchins usually amounted to 4–8% of the total, but in some urchins the number attained 15–20%. For example, on July 11 ten samples were examined from the stomach of a 51-mm. urchin that had fed for 10 hours. They contained a total of 522 individuals of *E. borealis*, of which 88 (nearly 17%) were judged by either of two criteria to be dividing: an elongated condition of the macronucleus or the presence of a transverse cytosomal constriction. Such specimens are easily recognized in living material, even with magnification as low as 10–20 ×, for *E. borealis* is a large ciliate, measuring on the average $143 \mu \times 87 \mu$ (Powers, 1933a). Many additional individuals were evidently preparing to divide, in view of their large size (length, 160–180 μ), and many had already divided, judged by the great variation in their size (length, 120–170 μ). (The individuals of stable, non-dividing populations of *E. borealis* are remarkably uniform in size.) Microscopic examination of some of the specimens showed that they had lost much of their transparency and contained great numbers of gastroles. It is a fact that this single urchin contained more dividing individuals of *E. borealis* than I had seen in all the hundreds of urchins examined in three earlier summers. In a 42-mm. urchin examined on August 16 after 15 hours of feeding, 20% of the individuals of *E. borealis* were dividing.

Period 3. Nearly half the urchins (48%) contained dividing specimens of *E. borealis*, but *M. indomita* remained non-divisional. However, the division of *B. gracilis* was resumed in 40% of the urchins.

TABLE I

Incidence of division of three species of ciliates in urchins (Strongylocentrotus droebachiensis) which were starved for two weeks and then fed generously on Laminaria during a 5-day experimental period

Successive 8-hour periods	No. of urchins examined per 8-hour period	Time in hours after beginning of feeding	Number (and percentage) of urchins in which ciliate indicated was dividing		
			<i>Entodiscus borealis</i>	<i>Madsenia indomita</i>	<i>Biggaria gracilis</i>
1	20	1–8	0	0	0
2	30	9–16	18 (60)	0	0
3	25	17–24	12 (48)	0	10 (40)
4	21	25–32	10 (48)	0	17 (81)
5	19	33–40	9 (47)	0	19 (100)
6	22	41–48	9 (41)	0	22 (100)
7	17	49–56	7 (41)	6 (35)	17 (100)
8	24	57–64	19 (79)	23 (96)	24 (100)
9	20	65–72	13 (65)	13 (65)	20 (100)
10	18	73–80	6 (33)	12 (67)	18 (100)
11	21	81–88	6 (29)	10 (48)	21 (100)
12	24	89–96	4 (17)	6 (25)	24 (100)
13	13	97–104	2 (15)	3 (23)	13 (100)
14	10	105–112	1 (10)	2 (20)	10 (100)
15	10	113–120	2 (20)	2 (20)	10 (100)

The first dividing specimens of *B. gracilis* appeared in one of four urchins that had fed for 21 hours. Of 12 urchins that had fed for 22, 23, or 24 hours, *B. gracilis* was dividing in nine. Although *B. gracilis* is also a fairly large ciliate in which dividing specimens are easy to recognize, the number of such specimens was never great in any urchin of the entire study; usually it amounted to 2–4% of the total. Evidently division is resumed in *B. gracilis* soon after the arrival of fresh food in the rectum.

Periods 4–6. The division of *E. borealis* continued in many of the urchins (41–48%), but *M. indomita* was still not dividing. In Period 4, dividing specimens of *B. gracilis* were present in 81% of the urchins; in Periods 5 and 6, and indeed in all subsequent periods, it was dividing in all the urchins.

In an ideal experiment the history of each of the ciliates should be followed in one and the same urchin by removing samples at intervals throughout the experiment. Unfortunately, this procedure is not feasible at present, and an urchin must be sacrificed at each examination. Such an examination may not reveal correctly the actual physiological condition of the ciliates with respect to division. For example, reference to Table I (Period 4, Column 4) shows that *E. borealis* was dividing in 10 of the urchins; it is understood that it was not dividing in the remaining 11 at the time of the examinations. But in some of the 11, there was great variation in size among the individuals and they contained many gastroles, suggesting that they had already divided at least once and were preparing for another division. Thus, in many urchins in which *E. borealis* was recorded as non-divisional, it was probably in a cycle of division and the percentages recorded in the table are actually low. These same comments apply to *M. indomita*, beginning with Period 7.

Period 7. This period is of special interest, in that dividing specimens of *M. indomita* made their first appearance in many of the urchins (35%).

The first dividing specimens of *M. indomita* appeared in a 49-mm. urchin that had fed for 50 hours, although they amounted to only 1% of the total. The division of *M. indomita* offered special difficulties of observation, since it is a slender, flattened, transparent ciliate, which is very active in samples of enteric fluid diluted with sea water for making counts. Magnifications high enough to reveal the condition of the macronucleus in living specimens (about 45 \times) render especially difficult the counting of specimens in samples. Therefore, the presence of a cytosomal constriction was used as the sole criterion of division and many pre-divisional specimens with elongated macronuclei were undoubtedly overlooked. The number of dividing individuals identified and recorded by the method never exceeded 5%.

Period 8. In this period the division of *E. borealis* and *M. indomita* attained its maximal incidence in the urchins (79% and 96%, respectively).

It is scarcely necessary to mention that population densities increased measurably in all the ciliates as division continued.

Periods 9–15. In these periods the percentage of urchins that contained dividing specimens of *E. borealis* and *M. indomita* gradually decreased, but with minor fluctuations which may have resulted from variations in the total numbers of urchins examined.

In urchins kept four days or longer with food, it is somewhat difficult to relate

satisfactorily the division of *E. borealis* and *M. indomita* to the amount of food in the gut, since many urchins stop feeding after three days and then feed irregularly. Sixteen urchins which had remained 9–14 days with food were examined. Two had dividing specimens of *E. borealis* and one had those of *M. indomita*. Conditions in these urchins probably approximated those found in urchins under natural conditions; that is, the amount of food in the gut was variable and the condition of the two ciliates was unpredictable, though usually non-divisional.

DISCUSSION

The results show conclusively that under certain experimental conditions (starvation of the host, followed by generous feeding) there is a direct relation between the feeding of the urchin and the division of its ciliates: starved urchins contain no dividing ciliates, whereas many urchins fed on laminaria contain great numbers. With reference to the time at which division is resumed in experimentally fed urchins, the results permit these conclusions: division is resumed in *B. gracilis* after 20–30 hours of feeding; a period of division is initiated in *E. borealis* after 10–15 hours of feeding; a similar period is initiated in *M. indomita* after 50–60 hours. The evidence indicates that division continues indefinitely in *B. gracilis*, provided any appreciable amount of food remains in the rectum. The duration of the period of division of *E. borealis* and *M. indomita* is difficult to determine, owing to irregularities in the feeding habits of the host; the evidence indicates that it continues for two to three days and then subsides in most of the urchins.

It is clear that the results cast serious doubt on my earlier postulate to the effect that cycles of division are inherent in *E. borealis* and *M. indomita*, though they do not actually disprove the existence of such cycles. Nevertheless, it seems more likely that the occasional outbreaks of divisional activity are associated with periods of generous feeding on the part of the host.

For the present the precise factors that are responsible for the division of the ciliates must remain unidentified. Presumably the presence of abundant food in the urchin gut results in a great increase in the numbers of bacteria that are both available and suitable as food for the ciliates. There is no doubt that the bacterial flora is greatly augmented by generous feeding on the part of the host. An examination of enteric fluid from a well-fed urchin reveals great numbers of bacteria, whereas fluid from a starved urchin contains relatively few. It is recognized that absolute numbers may be of little significance, for some ciliates are extremely selective in their ingestion of bacterial food. For example, Kidder (1941, p. 471) isolated from a mass culture of *Tillina canalifera* 26 types of bacteria, only one of which was suitable to maintain the growth of the ciliate. Nevertheless, the many gastroles in ciliates from well-fed urchins stand as proof of generous feeding, and the division of the ciliates has been conclusively demonstrated. Thus, it is clear that suitable bacteria were present in plentiful numbers, and it is assumed that division was the direct result of increased food ingestion. Whether the digestive juices of the urchin are important in stimulating division is unknown.

Until the dietary requirements of the three ciliates are conclusively established, the facts seem to permit the following interpretation of the normal urchin-ciliate relationship. Ingested material is nearly always present in the rectum of any urchin

collected in nature, and this material suffices to support a bacterial flora that is both adequate and suitable to maintain *B. gracilis* in a constantly dividing state. The amount of ingested material in the stomach and intestine of such an urchin is demonstrably variable. The flora that it supports is considerable, but usually the flora suffices merely to maintain *E. borealis* and *M. indomita* in a non-dividing state. Occasionally an urchin finds a plentiful supply of food and fills to repletion. Then the numbers of suitable bacteria increase to the extent that they are adequate to initiate and sustain a period of division.

SUMMARY

1. The study deals with division in the ciliates *Entodiscus borealis*, *Madsemia indomita*, and *Biggaria gracilis*. All the urchins collected in nature contained dividing specimens of *B. gracilis*, but only 3% of them contained those of *E. borealis* and *M. indomita*.

2. Urchins were kept without food for 2-3 weeks; in these, dividing specimens of the ciliates were absent.

3. After about two weeks of starvation, the urchins were supplied with generous amounts of the kelp *Laminaria*, and the percentage of the urchins that contained dividing specimens of each of the ciliates was recorded by successive 8-hour periods during 5 days of feeding.

4. Division began in *E. borealis* after 10-15 hours of feeding by the host, and dividing individuals were present for about three days in 33-79% of the urchins.

5. Division began in *M. indomita* after 50-60 hours, and dividing specimens were present for about two days in 25-96% of the urchins.

6. Division began in *B. gracilis* after 20-30 hours, and dividing specimens were present in all the urchins after the second day.

7. Division appears to continue indefinitely in *B. gracilis*, provided any appreciable amount of food is present in the urchin gut. Although division appears to occur discontinuously in *E. borealis* and *M. indomita*, it is doubtful that cycles of division are inherent in them, as postulated earlier. It seems more likely that their division is correlated with the copious ingestion of suitable bacteria, whose numbers are greatly increased by the presence of abundant food in the urchin gut.

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